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FOURIER TRANSFORM INFRARED SPECTROSCOPY IN TREATED WOODS DETERIORATED BY A WHITE ROT FUNGUS

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In memoriam of Dr. Thomas C. MANNES

ABSTRACT

This study aimed to analyze chemical changes by Fourier Transform Infrared Spectroscopy of *Eucalyptus dunnii* and *Pinus elliottii* treated woods subjected to an accelerated decay test with the white rot fungus *Ganoderma applanatum*. The wood test specimens (2,5x2,5x0,9 cm) were impregnated with preservative solutions of chromated copper borate and synthetic pyrethroids and carbamates with 6% concentration by a vaccum-pressure process. After a decay test of 16 weeks, the chemical changes of the treated and untreated wood samples were evaluated by Fourier Transform Infrared Spectroscopy. It was observed that the rot fungus attack caused a variation in the intensity and a displacement of spectrum peaks, indicating a change to the woods' chemical composition after fungal exposure. The lignin / carbohydrate ratio indicated that the fungus had no preference for a specific cell wall component, pointing to a simultaneous deterioration of cellulose, hemicellulose and lignin in the treated and untreated woods. Finally, the Fourier Transform Infrared Spectroscopy technique, together with the lignin / carbohydrate ratio analysis, proved efficient in the study of the variation of the wood chemical modifications deteriorated by rot fungi.

Keywords: Chemical change, *Eucalyptus dunnii, Ganoderma applanatum*, *Pinus elliottii*, wood preservation.

INTRODUCTION

One of the main requirements of the wood consumption market is quality. Even though wood presents several advantages over other materials, due to the wide possibility of uses, and because it is a heterogeneous material and is basically formed by carbon, it is a material susceptible to degradation by rot fungi, which compromises its use.

Many authors, e.g. Lima *et al.* (2014) emphasized that preservation aims to increase the useful life of the wood by protecting from deterioration caused by xylophages agents. Many other authors, e.g. Vidal *et al.* (2015) supplemented that the application of a preservative treatment is fundamental to increase wood durability in service. Such treatments make use of chemical produtcs classified as oily, oil soluble and water soluble (Galvão *et al.* 2004) and when applied cause modification to the chemical composition of the wood due to an interaction with the cell wall of that material (Lebow 2010 and Rowell 2014).

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Beyond the changes resulting from these products, when wood is exposed to favorable conditions for the development of degrading organisms, it suffers a break of chemical bonds in the polymers that compose it (cellulose, hemicellulose and lignin) caused by the action microorganism.

An effective way of analyzing recurring wood chemical modifications is by Fourier Transform Infrared Spectroscopy (FT-IR). Pandey (1999), Lopes and Fascio (2004), Alabarse (2009) and Popescu *et al.* (2010) reported that the chemical characterization of materials can be performed by this technique, since it considers the molecules' vibrational frequency.

Considering that each molecule vibrates at a specific wavelength, FT-IR spectroscopy becomes an important tool for the identification of organic components present in the wood. So much that Pandey and Pitman (2003), Costa *et al.* (2011), Fackler and Schwanninger (2012), Darwish *et al.* (2013), Yilgor *et al.* (2013) and Zhang *et al.* (2015), analyzed the recurrent modifications on the chemical composition of wood deteriorated by rot fungi.

However, in some cases, the large amount of information that may be present in the spectra ends up generating great difficulties for the appropriate interpretation of the chemical compounds (Lopes and Fascio 2004), as well as issues with overlapping bands, which limit the suitable identification of organic components.

To assist the analysis of changes to wood chemical components after fungal deterioration, authors such as Pandey and Pitman (2003) and Costa *et al.* (2011) used a relationship between the variations in the lignin and carbohydrate ratios, through the peaks of relative intensities in the bands of interest, which makes it possible to verify the deterioration mechanisms by the employed rot fungus.

As such, the objective of the present study was toqualitatively analyze the modifications in the chemical composition of preserved *Eucalyptus dunnii* and *Pinus elliottii* wood, submitted to deterioration by the white-rot fungus *Ganoderma applanatum*, using Fourier Transform Infrared Spectroscopy (FT-IR).

MATERIAL AND METHODS

Materials used

The specimens used measured 2,5x2,5x0,9 cm (tangential, radial, longitudinal) from the cutting of *Eucalyptus dunnii* Maiden and *Pinus elliottii* Engelm. boards, and were approximately 28 and 15 years old, respectively. After preparation, the specimens were placed in an air-conditioned room (65% humidity and 25 ° C temperature) until they reached a constant mass. After stabilization, the wood samples were impregnated with the preservative solutions.

Preparation of preservative solutions and wood impregnation

Two wood preservatives were used: a watersoluble product containing copper chromate borate (CCB), and the other oleosoluble based on synthetic pyrethroids and carbamates (PSC), both with a concentration of 6%. The concentration was selected based on recommendations by the product manufacturers.

Aiming to obtain the preservative solution, the watersoluble product was diluted in distilled water, while the oil soluble was diluted in acetone. The vacuum-pressure impregnation process was done in a laboratory autoclave with 10 cm of diameter and 30 cm of length. Initially, a vacuum inside the autoclave containing the wood samples was generated for a period of 15 minutes in order to withdraw the air, and subsequently, the autoclave was filled with the preservative solution, applying approximately a 8 bar pressure, for 90 minutes. After impregnation, the wood samples were placed in a laboratory stove, set at 50 ° C, to fix the chemical inside the wood.

Biological deterioration

The decay test was adapted from the D 2017 standard from the American Society for Testing and Materials (ASTM 2005) as well as an experiment performed by Modes *et al.* (2012). The treated and untreated wood samples were submitted to deterioration by the white rot fungus *Ganoderma applanatum* (Pers.) Pat., for 16 weeks period, which aimed to evaluate changes in the chemical composition of wood.

Qualitative analysis of the chemical composition

The FT-IR technique was used to accomplish a primary chemical components qualitative analysis (cellulose, hemicellulose and lignin) of the treated and untreated *E. dunnii* and *P. elliottii* woods submitted to deterioration by *G. applanatum*.

After exposure to the fungus, the specimens were milled in a Willey-type mill, passing through a set of sieves with 40 and 60 mesh, respectively, and the fraction used was the material retained in the 60 mesh.

The analysis was performed on a Perkin Elmer Fourier transform spectrometer, model Spectrum Two. The parameter considered was the transmittance, with readings in the region between 1800 and 800 cm^{-1}. The resultant spectrum of each treatment corresponded to the average of 32 scans, normalized in the 1030 cm^{-1} band (reference for wood sample analysis), grouped as a total spectrum.

Aiming to facilitate an understanding of the variations to the chemicals components of the woods subjected to deterioration, together with the spectra, and to increase the reliability of the results, a complementary analysis was developed from the chemical compounds' relative intensities variations.

890 cm^{-1} (cellulose), 1420 cm^{-1} (polyoses) and 1740 cm^{-1} (carbonyls) bands were observed, and the 1508 cm^{-1} band (lignin) was selected as a reference, since, according to Costa *et al.* (2011), it is characterized by high purity.

Statistical analysis

The statistical analysis was performed in the Statgraphics Centurion software, through variance analysis and later comparison of averages by Tukey test, with a 1% of error probability for the relative intensities.

RESULTS AND DISCUSSION

FT-IR analysis

Considering the wood samples, the FT-IR spectra analysis is better represented in the region between 1800 and 600 cm-1, commonly known as fingerprint. In this region, the well defined peaks of the main functional groups present in the wood structure were found (Pandey 1999). In this context, Popescu *et al.* (2010) supplemented that in regions where the predominant vibrations are of the O-H and C-H groups, among them the region between 1800 and 800 cm^{-1}, where each functional group vibrates at a well defined frequency, the spectrum intensity variation can then be attributed to the hydrogen bonding force.

Fackler and Schwanninger (2012) complement that chemical composition has been repeatedly studied through the vibrations in the functional groups O-H and C-H. Each specific grouping is associated to a band, thus enabling the characterization of the wood's cell wall components, cellulose, hemicellulose and lignin (Table 1).

Table 1. Characterization of bands and wood chemical components by infrared spectroscopy.

Wavelenght (cm^{-1})	Functional group / Vibration type	Chemical compound	Reference
1740	C=O bond of the carboxylic group	Hemicellulose	Zhang *et al.* (2015)
1592	Aromatic skeletal vibrations plus C=O stretch	Lignin	Pozo *et al.* (2006)
1508	C=C stretching vibration in aromatic ring	Lignin	Costa *et al.* (2011) / Darwish *et al.* (2013)
1460	Aromatic C–H deformation	Lignin	Fackler *et al.* (2007)
1420	$C–H_2$ scissor vibration	Cellulose	Tomak *et al.* (2013)
1370	C–H deformation	Cellulose/ Hemicellulose	Tomak *et al.* (2013)
1320	CH_2 rock vibration	Cellulose	Sun *et al.* (2015)
1260	C–O stretching in xylan	Hemicellulose	Darwish *et al.* (2013)
1230	Unconjugated C=O stretching in xylan	Hemicellulose	Yilgor *et al.* (2013)
1155	C–O–C stretching	Cellulose / Hemicellulose	Darwish *et al.* (2013)
1030	C–O stretching and C–H deformation in the guaiacyl unit	Cellulose / Hemicellulose / Lignin	Darwish *et al.* (2013)
890	C–OH stretching vibration	Cellulose	Zhang *et al.* (2015)

Figure 1 and Figure 2 present the FTIR transmittance spectra resulting from deteriorated *E. dunnii* and *P. elliottii* woods, respectively, before and after biological deterioration by *G. applanatum*.

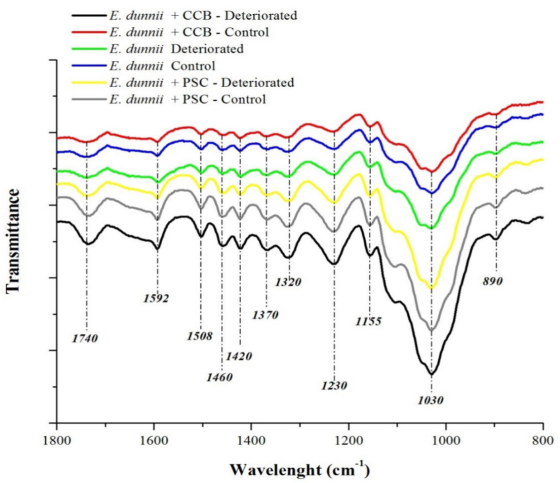

Figure 1. FTIR spectra of treated and untreated *Eucalyptus dunnii* wood deteriorated by the white rot fungus *Ganoderma applanatum*.

Considering Table 1, the bands and their respective assignments, Figure 1 shows that in the specters of the CCB and PSC treated woods, there are occurrences of increased intensities in the bands of the

treatments submitted to deterioration in relation to the respective control treatments. In relation to the control groups, a small variation in the specters intensitiesis observed, indicating a low modification of the wood's chemical compounds with the application of preservative products.

For the deteriorated woods, the highest variations are found in the 1508 cm^{-1}, 1460 cm^{-1}, 1420 cm^{-1}, 1370 cm^{-1}, 1320 cm^{-1}, 1230 cm^{-1} and 1030 cm^{-1} bands, which correspond to the following references in Table 1: lignin, lignin, cellulose, cellulose / hemicellulose, cellulose, hemicellulose, cellulose / hemicellulose / lignin, respectively. This indicates that the fungus used degraded the three wood polymers simultaneously. Many white rot fungi are characterized by not being selective of the wood cell wall components deterioration (simultaneous white-rot species), which corroborates with the findings in this study, however, fewer white-rot species cause preferential (selective) lignin degradation (Schmidt 2006, Pandey and Nagveni 2007 and Costa *et al.* 2011).

Yilgor *et al.* (2013) and Zhang *et al.* (2015) described that the occurring peak intensity variation is considered a good indicator of the recurrent change in chemical components. In turn, these changes are possibly linked to the modification and / or deterioration of cellulose, hemicellulose and lignin, as well as the bond strengths between the atoms.

According to Darwish *et al.* (2013), the formation of aldehyde groups resulting from the hydrolysis of the bonds in two glucopyranose rings causes an increase in intensity in the 1030 cm^{-1} band. Already according to Pandey and Pitman (2003), the increase in the intensities of lignin-related bands are associated with the metabolization of carbohydrates, and not with the content of this chemical compound variation.

Figure 2 presents the main intensity variations occurring in the spectra of the untreated control group and of the deteriorated woods. A reduction of the specters intensity was observed for the deteriorated wood in relation to the control treatment in all bands. In their studies, Costa *et al.* (2011) determined that the variations of peak intensities are directly associated to the degradation of the chemical components caused by the fungus, similarly to the present study.

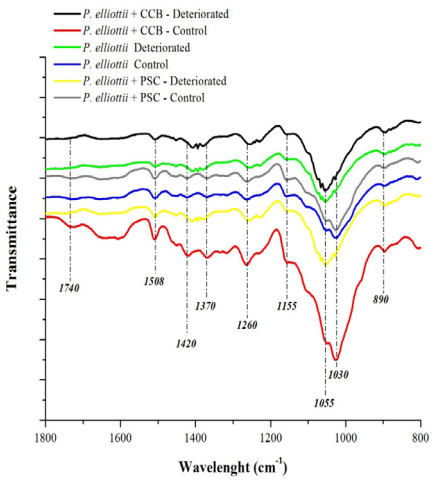

Figure 2. FTIR spectra for treated and untreated *Pinus elliottii* wood deteriorated by *Ganoderma applanatum*.

It is notable that a peak displacement from the 1030 cm^{-1} to the 1055 cm^{-1} band occurred for the deteriorated wood samples of all treatments, possibly due to deterioration and conversion of the wood cell wall compounds after the fungus action.

According to Backa *et al.* (2001), the variations of peak intensity and displacement are linked to

the appearance of new functional groups resulting from the degradation of cell wall components such as lignin, due to the action of oxidative processes from the enzyme action released by white-rot fungi.

In the case of the treated control samples, the intensity band variations may be associated to the interaction of the preservative compounds with the wood. In the case of water soluble preservatives, such as CCB, the presence of copper and chromium components make the solution highly reactive with wood, causing changes in its chemical structure (Lebow 2010). On the other hand, oil soluble preservatives, such as PSC, cause changes in the wood's cell wall composition, mainly by the adhesion of preservative acetyl groups replacing the wood hydroxyl groups (Rowell 2014).

However, considering the difficulty in the interpretation of some spectra, since overlapping bands may occur, thus limiting the correct identification of chemical compounds, the analysis by means of the relative intensity can be an effective alternative for the understanding of cellulose, hemicellulose and lignin variations within deteriorated woods.

Relative intensity

Table 2, Table 3, Table 4 present the relative intensities for the relationship between lignin and carbohydrates of treated and untreated *E. dunnii* and *P. elliottii* woods that were submitted to deterioration by *G. applanatum*. According to Table 2, a significant reduction in the lignin / carbohydrate ratio (I_{1508} / I_{1740}) was perceived in the *E. dunnii* control treatment. The CCB and PSC treatments did not present a significant difference in relation to its controls, indicating reduced modification of the wood cell wall component.

In the *P. elliottii* wood, a significant increase was observed in the I_{1508} / I_{1740} ratio of the deteriorated wood for both the control and PSC treatments, indicating a higher deterioration rate of cellulose and hemicellulose in relation to lignin. This was possibly associated with the wood's naturallylow durability and the interaction of the preservative with the structure of that material.

Table 2. Average values of lignin / carbohydrates (I_{1508} / I_{1740}) ratio of treated and untrated *Eucalyptus dunnii* and *Pinus elliottii* woods deteriorated by *Ganoderma applanatum*.

Treatment	Ratio I_{1508}/I_{1740}		*CV (%)*	*F*
	Control	Deteriorated		
E.dunnii	1,00573 a	1,00430 b	*0,08* *	*41,15* *
E.dunnii + CCB	1,00571 a	1,00641 a	*0,07* ns	*3,26* ns
E. dunnii + PSC	1,00634 a	1,00598 a	*0,07* ns	*0,52* ns
P. elliottii	1,00043 a	1,00192 b	*0,08* *	*173,23* *
P. elliottii + CCB	0,98403 a	0,99911 b	*0,80* *	*1350,15* *
P. elliottii + PSC	0,99941 a	0,99571 b	*0,20* *	*867,50* *

In that: Averages in the lines followed by the same letter, do not differ according to the Tukey test, in a 1% error probability; * = Significantly different at 1% error (P <0,01); ns = does not present significant difference with 1% of error (P> 0,01); CCB (chromated copper borate); PSC (synthetic pyrethroids and carbamates); QCC (copper chelate and carbamates).

Regarding the I_{1508} / I_{1420} ratio (Table 3), there was a significant difference in the majority of treatments for *E. dunnii* wood (the exception was the treatment based on PSC), and in all treatments concerning deteriorated *P. elliottii* wood. A significant increase was observed in the samples of the control treatment and for those treated with CCB, due to the higher degradation rate of cellulose and hemicellulose in relation to lignin in the *E. dunnii* wood, whereas a non-significant reduction for this reason was observed in the PSC treatment.

Table 3. Average values of lignin / carbohydrates ratio (I_{1508} / I_{1420}) of the treated and untrated *Eucalyptus dunnii* and *Pinus elliottii* woods deteriorated by *Ganoderma applanatum*.

Treatment	RatioI_{1508}/I_{1420}		*CV (%)*	*F*
	Control	**Deteriorated**		
E.dunnii	1,00147 a	1,00349 b	*0,12 **	*20,62 **
E.dunnii + CCB	1,00290 a	1,00963 b	*0,37 **	*87,34 **
E. dunnii + PSC	1,00792 a	1,00495 a	*0,20 ns*	*11,16 ns*
P. elliottii	0,99880 a	1,00170 b	*0,16 **	*142,91 **
P. elliottii + CCB	1,02024 a	1,00453 b	*0,82 **	*1591,39 **
P. elliottii + PSC	0,99962 a	1,00168 b	*0,12 **	*26,39 **

In that: Averages in the lines followed by the same letter, do not differ according to the Tukey test, in a 1% error probability; * = Significantly different at 1% error (P <0,01); ns = does not present significant difference with 1% of error (P> 0,01); CCB (chromated copper borate); PSC (synthetic pyrethroids and carbamates); QCC (copper chelate and carbamates).

Regarding the I_{1508} / I_{890} ratio (Table 4), considering the *E. dunnii* treatments, a significant increase was observed for the wood samples treated with CCB and a non-significant increase was recorded for the control group. This shows that the deterioration of cellulose was higher in relation to lignin. A significant reduction in this ratio was observed in the deteriorated specimens belonging to the CCB treatment of *P. elliottii* wood, with the control treatments and the PSC base showing non-significant variations.

Table 4. Average values of lignin / carbohydrates (I_{1508} / I_{890}) ratio of the treated and untrated *Eucalyptus dunnii* and *Pinus elliottii* woods deteriorated by *Ganoderma applanatum*.

Treatment	RatioI_{1508}/I_{890}		*CV (%)*	*F*
	Control	**Deteriorated**		
E.dunnii	0,98643 a	0,98813 a	*0,13 ns*	*8,74 ns*
E.dunnii + CCB	0,98746 a	1,00195 b	*0,78 **	*271,22 **
E. dunnii + PSC	0,99969 a	0,99260 b	*0,40 **	*47,90 **
P. elliottii	0,98463 a	0,98519 a	*0,06 ns*	*2,31 ns*
P. elliottii + CCB	1,01221 a	0,99125 b	*1,10 **	*2562,21 **
P. elliottii + PSC	0,98651 a	0,98622 a	*0,05 ns*	*0,83 ns*

In that: Averages in the lines followed by the same letter, do not differ according to the Tukey test, in a 1% error probability; * = Significantly different at 1% error (P <0.01); ns = does not present significant difference with 1% of error (P> 0.01); CCB (chromated copper borate); PSC (synthetic pyrethroids and carbamates); QCC (copper chelate and carbamates).

According to Costa *et al.* (2011), the decrease of the lignin / carbohydrate ratio is due to a preference of the rot fungus to deteriorate the lignin at a higher rate, in detriment of the other components, such as cellulose and hemicellulose.

However, in the present study, it was verified that the ratios showed a reduction and an increase in their values, possibly indicating a simultaneous deterioration of the chemical compounds of the wood, which corroborates with Schmidt (2006) and Pandey and Nagveni (2007), who highlight that in simultaneous white rot fungi, lignin and carbohydrates are removed at a similar rate, causing a homogeneous deterioration of the wood.

CONCLUSIONS

The white-rot fungus *Ganoderma applanatum* caused changes in the chemical compounds (cellulose, hemicellulose and lignin) of the *Eucalyptus dunnii* and *Pinus elliottii* wood, especially in not chemically trated samples.

The Fourier Transform Infrared spectroscopy (FT-IR) demonstrated to be an efficient tool for analyzing wood chemical composition variation, both for preservative treatments and to verify the influence of the deterioration caused by a fungus. Therefore, within the general context of this research, the chemical modification analysis through the lignin / carbohydrate ratio interpretation, together with the FTIR spectra, has become a valuable source of information about the deterioration mechanisms adopted by white-rot fungus *Ganoderma applanatum*.

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